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Effects of Fatty Acids and Vitamin E Supplementation on Behavioral Development of the Second Generation Rat

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Abstract

In this study, we examined the effects of dietary fatty acids on the fatty acid composition of phospholipid fractions in regions of the brain and on behavioral development in rats. The Sprague Dawley rats were fed the experimental diets 3~4 wks prior to the conception. Experimental diets consisted of 10% fat(wt/wt) which were from either safflower oil (SO, poor in ω3 fatty acids), mixed oil (MO, P/M/S ratio = 1:1.4:1, ω6/ω3 ratio = 6.3), or mixed oil supplemented with vitamin E (+500 mg/kg diet). At 3 and 9 weeks of age, frontal cortex (FC), corpus striatum (CS), hippocampus (H), and cerebellum (CB) were dissected from the whole brain. The fatty acid content was determined in the different phospholipid fractions: phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylethanolamine (PE) in the rat brain regions. In the visual discrimination test, the order of the cumulative errors made in Y-water maze test were SO > MO > ME. This suggested that the balanced diet supplemented with vitamin E had the most beneficial effect on learning ability. The overall characteristics of correlation between fatty acids and behavior development were that the frequency of cumulative errors were negatively correlated significantly with monounsaturated fatty acids (MUFAs), ie., 18:1 ω9 and 22:1 ω9. Docosahexaenoic acid (22:6ω3) of PS in frontal cortex (FC) was negatively correlated with the number of errors made in the Y-water maze test. 22:5ω6 PS in hippocampus (H), PC and PE in corpus striatum (CS), PC in cerebellum (CB) were positively correlated with cumulative errors. And these errors were negatively correlated with 20:4ω6 of PE in corpus striatum (CS) and PC in cerebellum (CB). Especially, Oleic acid (18:1ω9) in all phospholipid fractions (PC, PS, PE) of hippocampus was negatively correlated with the number of errors. These findings demonstrate that the MUFAs were might be essential for proper brain development, especially in hippocampus which is generally thought to be the regions of memory and learning.

Key words: fatty acids, vitamin E supplementation, DHA, AA, ω3/ω6 fatty acids, 18:1 ω9, behavioral development

INTRODUCTION

Docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (AA, 20:4ω6) are the predominant ω3 and ω6 polyunsaturated fatty acid (PUFA), respectively, in the mammalian central nervous system (1). Large amounts of ω3 and ω6 fatty acids are needed for membrane lipid synthesis during growth and development (2). Deficiency of ω3 fatty acid leads to a decrease in the DHA levels of brain and retina, thereby causing an abnormal state of both visual reaction for humans (3) and retinal function for animals, and impaired cognitive ability (4). According to the report from Yeh et al. (5), it has been shown that by feeding safflower oil deficient in ω3 fatty acid during lactation to a mother mouse, the DHA level within the cerebral tissue of an infant mouse was lower than when soybean oil was fed, and the searching ability decreased; and that when the safflower oil was replaced with soybean oil after the weaning period, the DHA level of the cerebral tissue was restored to the normal state, but the searching ability was not restored to the normal state. Delion et al. (6) reported that there was a change in dorpaminergic and serotonergic neurotransmitting states in frontal cortex involved in attention and cognitive process when the intake of 18:3ω3 was deficient for a long time.

Wainwright et al. (7) reported that the weight of animals with a diet deficient in EFA was 70% of the control group, while there was no difference in swimming speed. Their subsequent studies showed, however, that the deficiency of ω3 fatty acid had no effect on the memory and cog-

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nitive ability in the Morris water maze (8). Furthermore, Um's research (9) also showed that the fatty acid composition of synaptosomes, among brain subcellular fractions, was especially highly correlated with learning ability. Youdim et al. (10) reported that neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, which are characterized by loss of cognitive function resulting from aging, are associated with decreased AA and DHA, PUFA of cerebral tissue, and that restoring proper balance of $\omega 6/\omega 3$ fatty acids could slow progression to these disorders.

Several studies have been recently carried out to investigate the correlation of 18:1 $\omega 9$ to behavioral development, because it is known to be an important fatty acid in the process of myelination. Guan et al. (11) reported that there was a decrease of 20% in the amount of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in hippocampus and frontal cortex of brain sections in brains from Alzheimer's disease patients, and they suggested the correlation of 18:1 $\omega 9$ to cognitive function by reporting that 18:1 $\omega 9$ in PE had become significantly lower than normal. A study on the effect of the level of dietary vitamin E on behavior presented a contrary report. Lal et al. (12) reported that when a diet deficient in vitamin E was fed, the cognitive ability declined while the motor ability was not affected, and that addition of vitamin E to a diet had no effect on the memory and learning ability (13). However, other studies have had contradictory results, demonstrating that mice administered vitamins E and C for four to five months had their cognitive functions were improved (14).

This study examined the effects of diets with either desirable concentrations of $\omega 6/\omega 3$ and P/M/S (mixed oil-fed group) with or without vitamin E, or deficient in $\omega 3$ series fatty acids (safflower oil-fed group) on the fatty acid composition of brain sections and on behavioral development.

MATERIALS AND METHODS

Animals and diets

Female Sprague-Dawley rats were fed the experimental diets 3~4 weeks prior to conception and during pregnancy. Maternal experimental diets consisted of 10% fat (by weight), which was either safflower oil poor in $\omega 3$ fatty acid or mixed oil with P/M/S ratios, 1/1.4/1, $\omega 6/\omega 3$ ratio, 6.3/1. This mixed oil with the desirable ratio of P/M/S and $\omega 6/\omega 3$ was chosen from various combinations of oil generated by a self developed computer program. The mixed oil consisted of 1.8% corn oil (Sam Yang Co., Ltd. Seoul, Korea), 0.5% soybean oil, 4.5% palm oil, 0.5% menhaden (Zaphata Haynie Corp. USA), 2.5% canola oil (Nong Shim Co. Ltd. Seoul, Korea), and arachidonic acid 0.2%. The composition of maternal experimental diets and their fatty acid contents are shown in Table 1. Vitamin E levels of all diet were 50 mg α-tocopherol acetate/kg diet. In ME (MO + vitamin E) group, 500 mg α- tocopherol acetate/kg diet was supplemented.

Rat pups were fed the same diets with maternal diet and sacrificed at 3 and 9 weeks of age to measure fatty acid composition of brain sections and the Y-maze test at 9 weeks.

Dissection of rat brain regions

The rat brain regions were dissected out by cutting the head without anesthesia and frontal cortex (FC), hippocampus (H), corpus striatum (CS) and cerebellum (CB) were obtained by dissecting out from the whole brain on ice.

Phospholipid fractionation and fatty acid analysis of brain sections

The lipids of brain fractions were extracted according

Table 1. Composition of experimental diets (%)

Ingredient	Experimental groups		
	Safflower oil (SO)	Mixed oil (MO)	MO+Vit E (ME)
Carbohydrate[1]	65.0	65.0	65.0
Mixed oil[2]	-	10.0	10.0
Safflower oil	10.0		
Others[3]	25.0	25.0	25.0
18:2 $\omega 6$[4]	77.7	24.1	24.1
18:3 $\omega 3$	ND[5]	2.5	2.5
20:4 $\omega 6$	0.2	0.6	0.6
20:5 $\omega 3$	ND	0.7	0.7
22:6 $\omega 3$	ND	0.5	0.5
Total $\omega 6$	78.0	24.66	24.66
Total $\omega 3$	-	3.9	3.9
Total $\omega 6/\omega 3$		6.3	6.3
P[6]	78.0	28.1	28.1
M	9.1	40.0	40.0
S	11.4	28.0	28.0
P/M/S	6.9/0.8/1	1.1/1.4/1	1.1/1.4/1
α-tocopherol acetate supplementation (mg/kg diet)	-	-	500

[1]Starch : sucrose = 80 : 20.
[2]The mixed oil (10% by wt) consisted of 1.8% corn oil, 0.5% soybean oil, 4.5% palm oil, 0.5% menhaden, 2.5% canola oil, arachidonic acid 0.2% (This mixture was selected from the computer-searched combinations of various fats and oils for this study).
[3]Others contained 17.9% casein, 0,1% DL-methionine, 4% salt mixture (AIN-76, ICN, USA) and 1% vitamin mixture (AIN-76, ICN, USA) and 2% carboxymethyl cellulose.
[4]Values are expressed as relative % of total fatty acid.
[5]ND: Not detected.
[6]P: Polyunsaturated fatty acids, M: Monounsaturated fatty acids, S: Saturated fatty acids.

to the method of Folch (15). The phopholipids were separated by thin layer chromatography (TLC). The solvents were hexane : diethyl ether : acetic acid (80:20:2, v/v). The phopholipid fraction (PC,PS,PE) were separated by 2-dimensional TLC. The ratios of solvent for the phospholipid fractions were chloroform: methanol: acetic acid: H_2O, 100: 95:7:4 (volume ratio).

The silica gels were scraped off the plates immediately after TLC procedure and methylated by procedure of Lepage (16). The compositions of fatty acid esters were than determined by gas liquid chromatography (GLC, Hewlett-Packard 6890A). For the gas chromatographic separation, a bonded fused silica capillary column (OmegawaxTM 250) was used. The oven temperature of GLC was 180°C. The temperature of injection and detector ports was 280°C. Helium was used as the carrier gas for the column at a flow rate of 0.8 mL/min with a split ratio of 10:1. Fatty acid methyl ester standard was GLC reference standards (#GLC-87A), OmegawaxTM test min (#4-8576), PUFA-2 (#4-7015), Supelco, Bellefonte, PA, USA.

Visual discrimination test

A visual discrimination test in the Y-maze at 9 weeks of age was carried out. The procedure was derived from of Lamptey and Walker (17) and slightly modified by our laboratory (18). the Y-water maze consisted of an escape platform and two gates, one white and one black. The animals were trained in the maze for four days prior to the test period. On the first and second days, the rat was permitted to enter the white gate, where the escaped platform was located (positive response), or to enter the black gate, where no escaped platform was placed (negative response). The animal was permitted six positive and four negative runs in each of the 10-trial training sessions on the first two days. On the third and fourth training days, four trial runs were conducted and the animals were corrected for their wrong responses. During the following six consecutive days, each rat was permitted six trials a day and the negative trials were recorded as incorrect responses, ie., errors. The mean of the period were employed in comparing performances among different experimental groups. 30 rats per each group were tested in the Y-water maze.

Statistics

Statistical analysis was done using SAS procedure. The results of fatty acid analyses were presented as mean± SEM and analyzed by one-way analysis of variance (ANOVA). The results of Duncan's Multiple Test were presented and Pearson's Correlation Analysis was performed and results were presented with p-values.

RESULTS AND DISCUSSION

Fatty acids composition of brain section

Table 2 shows the polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) composition of the phopholipid fraction in the brain sections.

At the age of 9 weeks, the concentrations of SFA decreased and those of MUFA tended to increase in all brain regions compared to the values at 3 weeks because oleic acid (18:1 ω 9) increased due to active formation of myelin during lactation.

At the age of 3 weeks, the level of PUFA (polyunsaturated fatty acids) was significantly lower in SO group than MO group in PC of frontal cortex (FC), and significantly higher in the ME group than in the MO group in PC of hippocampus (H). The levels of PUFA for PC, PS, PE at the age of 9 weeks were not significantly different among 3 experimental groups in all rat brain regions.

MUFA (monounsaturated fatty acids) levels showed the tendency of SO > MO > ME. At the age of 3 weeks, the ME group had significantly higher level of MUFA for PE in frontal cortex (FC), corpus striatum (CS), and PS in cerebellum (CB) than the MO group. At the age of 9 weeks, the MUFA levels of PC and PS in hippocampus (H) of the SO group showed a significant decrease compared to MO group; MUFA levels between SO and ME group showed significant differences in PS of frontal cortex (FC), and PC of corpus striatum (CS).

In contrast to the level of MUFA, the overall tendency of SFA (saturated fatty acids) levels were SO > MO > ME. In SO group, the SFA levels in PE of frontal cortex (FC) at the age of 3 weeks were significantly higher than MO group. At the age of 9 weeks, SFA level of PC in corpus striatum (CS), and PC and PS in hippocampus (H) in SO group were significantly higher than in MO group.

Table 3 shows the level of 18:1 ω 9 of the phospholipids which is the major MUFA. The overall tendency of 18;1 ω 9 levels were SO < MO < ME. In ME group, 18:1 ω 9 levels of PE in frontal cortex (FC) & corpus striatum (CS), and PS in cerebellum (CB) of ME group at the age of 3 weeks showed significant increase compared to MO group. At the age of 9 weeks, there were significant differences in 18:1 ω 9 levels between the SO and ME groups in PC, PS, PE of the hippocampus (H) (Table 3).

In brain tissues, fatty acid synthesis takes place in oligodendrocytes, which synthesize a considerable amount of myelin lipid. It was suggested that these fatty acids were not affected by dietary fatty acid composition (19). In contrast to this result, it was reported that myelination in fish oil-fed rats was impaired (20). It was also reported that in the brain of mice fed diets high in fish oil there was a decline in the level of myelin basic protein, and that the activity of 2′,3′-cyclic nucleotide 3′-phophodiesterase, an enzyme used as a myelination indicator, was reduced (21). It has been also shown that 18:1 seems to protect against

Table 2. Effect of dietary fat and vitamin e supplementation on the polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) of the phospholipids in the rat brain regions at the age of 3 and 9 weeks

			3rd week			9th week		
			SO	MO	ME	SO	MO	ME
PUFA	FC	PC	21.71±0.92[1)b2)]	26.77±1.24[a]	26.30±1.69[a]	22.14±1.70	22.61±2.00	26.11±2.35
		PS	23.22±0.78	27.61±2.61	28.53±3.26	22.98±2.27	26.39±3.29	23.91±1.45
		PE	25.81±1.46	31.45±4.11	32.81±1.07	26.37±1.74	31.75±1.63	28.23±2.30
	CS							
		PC	23.57±1.55	25.98±3.06	22.11±4.03	23.12±2.60	29.21±2.57	25.76±3.28
		PS	25.51±2.14	26.03±1.03	22.76±1.07	25.63±2.27	25.44±3.09	29.16±1.91
		PE	26.06±3.42	33.31±3.21	32.65±3.28	26.48±2.84	30.62±2.01	32.17±0.55
	H							
		PC	18.84±2.15[b]	18.83±0.89[b]	21.57±2.89[a]	15.45±1.92	25.49±1.57	27.96±8.84
		PS	20.36±1.75	19.74±1.61	21.26±2.25	22.99±2.05	19.78±2.13	16.26±1.55
		PE	21.04±2.24	25.53±0.68	20.65±1.83	20.07±2.24	23.44±1.59	21.28±2.68
	CB							
		PC	30.2 ±3.92	27.32±3.42	32.42±3.98	27.42±0.98	30.42±4.32	27.34±3.43
		PS	24.86±1.80	31.09±2.70	27.55±2.98	26.63±3.15	28.40±1.35	23.19±2.80
		PE	25.80±1.52	28.43±2.73	31.16±2.27	28.03±2.56	31.88±2.13	27.85±2.12
MUFA	FC	PC	17.12±1.53	19.28±0.46	20.22±2.18	15.05±1.64	19.07±3.11	22.40±1.60
		PS	13.89±1.50	14.26±1.61	15.20±2.89	19.71±1.66[b]	23.25±1.08[ab]	27.39±2.99[a]
		PE	15.58±0.86[b]	16.07±2.12[b]	20.48±1.98[a]	32.10±2.87	27.87±2.76	24.64±0.92
	CS							
		PC	19.64±1.46	19.49±2.33	20.23±2.48	16.30±1.92[b]	21.19±2.46[ab]	23.14±1.29[a]
		PS	19.28±2.01	16.37±0.99	18.60±0.98	20.15±2.58	22.00±2.10	18.94±1.44
		PE	16.72±0.54[ab]	13.34±1.31[b]	22.51±3.24[a]	21.47±0.56	19.81±0.77	24.96±1.71
	H							
		PC	16.49±1.13	19.64±1.88	16.49±1.21	18.55±1.20[b]	24.86±2.80[a]	24.32±1.42[a]
		PS	13.69±0.44	19.59±2.53	14.28±3.35	20.89±2.39[b]	29.09±2.05[a]	27.18±1.88[a]
		PE	20.64±4.49	14.25±1.46	20.49±2.33	15.47±1.74	16.76±1.64	15.42±4.76
	CB							
		PC	16.75±0.90	14.13±1.65	17.17±0.72	20.00±2.06	16.91±6.14	21.48±1.79
		PS	12.22±1.33[b]	11.79±0.89[b]	17.58±0.11[a]	18.79±1.48	20.02±3.17	22.22±2.43
		PE	18.53±1.06	15.57±1.26	18.34±2.17	14.30±1.82	16.26±5.88	21.27±2.32
SFA	FC	PC	54.10±1.69	51.11±1.00	50.80±4.21	53.75±3.23	55.73±2.91	45.71±2.67
		PS	55.17±1.70	53.94±2.99	47.97±4.11	48.36±3.59	48.11±3.19	47.59±3.26
		PE	57.45±0.02[a]	47.87±4.01[b]	42.19±1.26[b]	42.12±1.74	44.23±3.38	47.74±2.17
	CS							
		PC	51.13±3.11	50.21±4.13	54.87±5.66	55.93±2.71[a]	45.59±3.62[b]	44.04±4.76[b]
		PS	48.14±1.35	53.14±2.17	52.57±1.65	43.06±1.72	43.14±2.41	45.87±1.59
		PE	48.51±1.69	48.44±2.66	43.36±1.49	42.93±2.89	44.28±1.98	43.56±0.96
	H							
		PC	58.47±3.19	55.59±1.00	57.24±4.19	60.06±2.91[a]	45.64±2.67[b]	45.99±7.03[b]
		PS	60.45±2.13	55.17±5.51	52.20±2.07	60.02±2.80[a]	50.77±2.56[b]	53.47±2.10[ab]
		PE	54.52±7.30	57.73±1.11	57.15±1.32	54.90±4.56	56.14±1.32	48.43±4.49
	CB							
		PC	50.32±3.81	55.91±1.38	48.18±2.62	49.20±2.64	49.72±4.47	49.53±3.26
		PS	56.92±1.54	48.34±4.45	51.93±2.34	47.06±2.53	47.64±3.46	51.32±2.22
		PE	46.24±2.93	51.62±3.14	50.97±3.41	47.70±1.61	44.95±4.25	46.23±2.23

FC: Frontal cortex, CS: Corpus striatum, H: Hippocampus, CB: Cerebellum.
SO: Safflower oil, MO: Mixed oil, ME: MO+vitamin E.
[1)]Values expressed as the wt% of total fatty acids. Mean±S.E.M.
[2)]Values with the different letters are significantly different from the others within the same row ($p<0.05$).

lipoperoxidative damage because MUFA is less sensitive than PUFA to peroxidative process (22).

Behavioral development

Visual discrimination in Y water maze : Fig. 1 shows the results of the visual discrimination tests at the age of 9 weeks. The test was performed 6 times per day for 6 days. In the visual discrimination test, the order of the cumulative errors made in Y-water maze test were SO > MO > ME. There was significant difference between SO group and ME group. In another rat study (23), long chain PUFA in diet was positively correlated with learning ability, and in a human study, Uauy et al. (24) suggested that

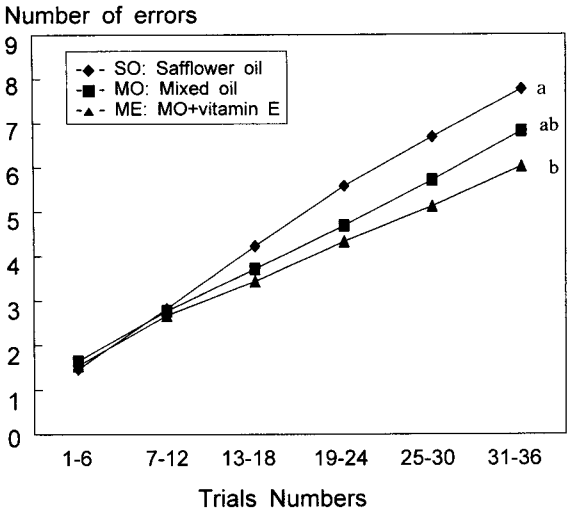

Fig. 1. Cumulative error on the visual discrimination test at the age of 9 weeks in the Y-water maze.

22:6 ω3 had an positive effects on photoreceptor membrane related to signal transduction processes, rhodopsin activation, and development of rod and cone. When the 22:6 ω3 was supplemented to preterm infants, the visual function level was similar to breast-fed preterm infants. The results in studies of term infants are controversial. Some researchers have suggested that 22:6 ω3 supple mented formula had a desirable effects on behavior (25, 26), and in rat studies, 22:6 ω3 ethyl ester supplementation improved brain function and behavior (27). However, in contrast to those results, some studies suggested that 22:6 ω3 interfered with behavioral development (18,28). Lee and Kim (18) reported that a fish oil fed group had reduced learning ability compared to a corn oil fed group. They suggested that this observation resulted from oxidation due to feeding a 20:5 ω3 and 22:6 ω3 rich diet for a long term period. Lamptey and Walker (17) observed that a safflower oil group showed worse learning ability than a soybean oil group when they fed to white rats with safflower oil which was lacking in ω3 fatty acids, and 18:3 ω3 containing soybean oil for two generations. Yamamoto et al. (29) suggested that 18:3 ω3 containing perilla oil had a positive effect on various abilities, including learning ability, as compared to 20:5 ω3 and 22:6 ω3 containing fish oil. On the other hand, there was a study in rats which concluded that the level of fatty acids in brain tissue was not associated with memory and learning ability (8).

Correlation between brain phospholipid fatty acids and behavior : Table 4 shows the correlation between the frequency of cumulative errors at the age of 9 weeks and fatty acid compositions of phospholipid in rat brain regions.

As for the correlation between cumulative errors and MUFA, the frequency of cumulative errors was negatively correlated significantly with 18:1 ω9 of PC in frontal cortex (FC) ($p<0.01$), PC ($p<0.05$), PS ($p<0.05$) and PE ($p<0.05$) in hippocampus (H), and PS in cerebellum (CB) ($p<0.01$). In addition, the frequency of cumulative errors was negatively correlated with 22:1 ω9 of PE in frontal cortex (FC) ($p<0.05$), that of PC in hippocampus (H) ($p<0.05$)

Table 3. Effect of dietary fat and vitamin E supplementation on oleic acid (18:1) of the phospholipids in the rat brain regions at the age of 3 and 9 weeks

		3rd week			9th week		
		SO	MO	ME	SO	MO	ME
FC							
	PC	11.54±1.52[1)]	14.07±0.53	13.45±2.54	11.10±0.60	13.08±3.12	16.71±1.04
	PS	9.74±1.06	9.41±0.89	10.40±2.78	15.21±1.59[a]	19.27±0.14[ab]	21.46±2.88[a]
	PE	10.61±1.47[b2)]	11.03±1.20[b]	15.45±0.87[a]	19.39±2.64	21.17±3.00	19.31±1.11
CS							
	PC	13.37±0.77	14.45±1.53	16.01±2.13	13.23±1.17	15.86±3.04	15.76±0.49
	PS	13.92±1.74	12.19±0.61	15.15±0.76	16.04±1.81	17.86±2.03	15.04±1.57
	PE	11.40±0.7[b]	12.81±1.21[b]	16.85±3.10[a]	16.57±1.07	16.16±0.83	17.31±1.64
H							
	PC	13.07±1.17	12.62±2.14	12.23±1.38	14.58±0.68[b]	20.09±2.16[a]	18.42±1.74[a]
	PS	10.99±1.25	15.49±2.17	12.22±3.20	14.22±1.83[b]	18.47±2.08[a]	19.18±2.30[a]
	PE	14.34±4.33	14.08±0.38	17.64±2.44	12.68±1.50[b]	14.03±1.68[ab]	17.46±5.01[a]
CB							
	PC	12.31±1.22	10.42±1.11	14.23±0.46	14.63±1.37	13.19±3.79	16.92±2.49
	PS	7.41±0.39[b]	9.64±1.58[b]	12.73±0.69[a]	14.96±0.84	14.53±2.03	16.11±2.11
	PE	15.37±0.81	11.61±1.37	15.58±1.31	10.55±1.71	12.52±5.97	16.18±1.35

FC: Frontal cortex, CS: Corpus striatum, H: Hippocampus, CB: Cerebellum.
SO: Safflower oil, MO: Mixed oil, ME: MO+vitamin E.
[1)]Values expressed as the wt% of total fatty acids. Mean±S.E.M.
[2)]Values with the different letters are significantly different from the others within the same row ($p<0.05$).

Table 4. Correlation coefficients between fatty acid compositions of the phospholipid in the brain regions and Y-water maze test at the age of 9 weeks

	FC			H			CS			CB		
	PC	PS	PE	PC	PS	PE	PC	PS	PE	PC	PS	PE
18:1	-.7228**	-.2737	.2387	-.6873*	-.6758*	-.5568*	-.0096	-.4787	-.2174	-.0685	-.7844**	-.3622
18:2 ω6	.1906	.7603**	-.0484	-.4004	-.1767	-.1594	.0441	.2986	.0714	-.0552	-.2319	.2237
18:3 ω6	.1103	.2145	.6604*	-.5529	-.2179	-.4815	.0179	-.1185	-.0525	-.1606	-.0731	-0562
18:3 ω3	-.4214	-.6613*	-.4995	-.2959	.3541	.0432	.2339	.5805	-.1177	-.1178	.5214	-.5151
20:4 ω6	-.2289	.2129	.1996	.1552	.3921	.2635	.1734	.0954	-.5360*	-.5896*	.1399	.0232
20:5 ω3	.0625	-.1032	-.2484	-.2599	.6191	-.5248	.2711	-.5421*	.2453	-.0474	.3086	-.1337
22:1	.3078	.1363	-.5916*	-.6015*	-.3279	-.0052	-.7945***	.5540*	-.2202	.4574	-.2119	.1123
22:5 ω6	-.3427	.0766	.2771	-.0962	.7345*	-.0525	.5990*	.1676	.6647*	.6979*	.5165	-.3494
22:6 ω3	-.0590	-.6582*	.2449	-.3029	-.3916	-.3264	-.2934	-.3581	-.2411	.0470	-.0823	-.1842
PUFA	.0101	-.1973	-.2658	-.3721	.6223*	-.1199	-.0733	.0724	-.5258*	-.2348	.2906	.0561
MUFA	-.4314	-.1931	.2455	-.1985	-.6601**	-.5245*	-.3359	-.5576*	-.2261	-.1911	-.7782**	-.3545
SFA	.3915	.5015	-.0346	-.5115	.2877	.4406	.3660	.4961	.3312	-.0763	.3505	.4686
ω6/ω3	.0610	.4894	.4943	.6433**	.4210	.4314	.4405	.2254	.1464	-.5374*	-.1136	.3271

Correlation coefficient are presented with p-value: *$p<0.05$, **$p<0.01$, ***$p<0.001$.
FC: Frontal cortex, CS: Corpus striatum, H: Hippocampus, CB: Cerebellum.
SO: Safflower oil, MO: Mixed oil, ME: MO+vitamin E.

and PC ($p<0.001$) and PS ($p<0.05$) in corpus striatum (CS). Therefore, the higher the level of MUFA (18:1 and 22:1) in brain phospholipids, the lower the frequency of errors.

As for the correlation between cumulative errors and ω3 fatty acids, cumulative errors were negatively correlated with 18:3 ω3 of PS in frontal cortex (FC) ($p<0.05$) and corpus striatum (CS) ($p<0.05$) and 20:5 ω3 of PS in corpus striatum (CS) ($p<0.05$), and 22:6 ω3 of PS in frontal cortex (FC) ($p<0.05$). As for the ω6 fatty acids, the frequency of errors showed significantly positive correlation with 18:2 ω6 of PS in frontal cortex (FC) ($p<0.05$) and 18:3 ω6 of PE in frontal cortex (FC) ($p<0.05$). As for 20:4 ω6, the frequency of errors was negatively correlated with PE in corpus striatum (CS) ($p<0.05$) and PC in cerebellum (CB) ($p<0.05$). Furthermore, the frequency of errors showed positive correlation with 22:5 ω6 of PS in hippocampus (H) ($p<0.05$), PC and PE in corpus striatum (CS) ($p<0.05$) and PC in cerebellum (CB) ($p<0.05$).

The overall characteristic of correlation between fatty acids and behavior development was that the frequency of cumulative errors was negatively correlated significantly with MUFA such as 18:1 ω9 and 22:1 ω9. Especially, the correlation between the cumulative errors and 18:1 ω9, which is known to be an important fatty acid for the myelination process and behavior development, should be noted. Guan et al. (11) found that the level of 18:1 of PE in brain tissues with Alzheimer disease was significantly lower than control. Prasad et al. (30) observed similar results, noting that the level of 18:1 of PE in hippocampus was decreased in Alzheimer's patients, suggesting the correlation between 18:1 and cognitive function.

Um et al. (9) investigated the correlation between the fatty acids in brain subcellular fraction and cumulative error, and concluded that 22:5 ω6 and 18:2 ω6 were positively correlated with the frequency of errors. These observations were similar to ours showing that the frequency of cumulative errors are significantly positively correlated with 22:5 ω6 and 18:2 ω6 of PS and PE in the frontal cortex (FC).

Vitamin E supplementation and behavioral development: The result that vitamin E supplementation had positive effects on learning ability might be due to the changes in membranes by vitamin E. It could be concluded from the results that vitamin E affected eicosanoid synthesis in plasma and platelets (31). Another possibility is that the difference in learning behavior by dietary fatty acids or vitamin E supplementation might be due to the changes in prostaglandins. Although Martin et al. (32) suggested that dopamine release was increased in striatum by vitamin E supplementation, Shukitt-Hale et al. (33) found that the deterioration of psychomotor function could not be delayed by vitamin E supplementation in old rats over the age of 18 months. Therefore, further research is needed to verify the correlation between vitamin E and behavior development.

SUMMARY AND CONCLUSION

In this study, we examined the effects of dietary fatty acids on the fatty acid composition and antioxidant systems of phospholipid fractions in the rat brain regions, and on behavioral development. The Sprague Dawley rats were fed the experimental diets 3~4 wks prior to the conception. Experimental diets consisted of 10% fat (wt/wt) which were safflower oil (SO, poor in ω3 fatty acids), mixed oil (MO, P/M/S ratio = 1:1.4:1, ω6/ω3 ratio = 6.3), and

mixed oil supplemented with vitamin E (+500 mg/kg). At 3 and 9 weeks of age, frontal cortex (FC), corpus striatum (CS), hippocampus (H), cerebellum (CB) were dissected out from the whole brain. Fatty acids were measuremed in different phospholipid fractions phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) in the rat brain regions. Visual discrimination test in Y-water maze at the 9 weeks of age were carried out.

At the age of 9 week, the concentrations of SFA decreased and those of MUFA tended to increase in all brain regions compared to the values at 3 weeks because oleic acid (18:1 ω 9) increased due to active formation of myelin during lactation. Furthermore, 18:1 ω 9 was higher in ME group than MO group.

In the visual discrimination test, the order of the cumulative errors made in the Y-water maze test were SO > MO > ME. This suggested that a balanced diet supplemented with vitamin E has the most beneficial effect on learning ability.

As for the correlation between frequency of errors and ω 3 and ω 6 fatty acids in brain phospholipids. cumulative errors were negatively correlated with 18:3 ω 3 of PS in frontal cortex (FC) ($p < 0.05$) and 20:5 ω 3 of PS in corpus striatum (CS) ($p < 0.05$), and 22:6 ω 3 of PS in frontal cortex (FC) ($p < 0.05$). And, these errors showed significantly positive correlation with 18:2 ω 6 of PS in frontal cortex (FC) ($p < 0.01$) and 18:3 ω 6 of PE in frontal cortex (FC) ($p < 0.05$). As for 20:4 ω 6, the frequency of cumulative errors was negatively correlated with PE in corpus striatum (CS) ($p < 0.05$) and PC in cerebellum (CB) ($p < 0.05$). These errors showed positively correlated with 22:5 ω 6 of PS in hippocampus (H) ($p < 0.05$), PC and PE in corpus striatum (CS) ($p < 0.05$), PC in cerebellum (CB) ($p < 0.05$). Especially 18:1 ω 9 in PC of frontal cortex (FC) showed a strong negative correlation with cumulative errors ($p < 0.01$), and 18:1 ω 9 in all phospholipid fractions (PC, PS, PE) of hippocampus (H) showed negative correlation with cumulative errors ($p < 0.05$), indicating that MUFAs might be indicator for brain development.

Further research is needed to clarify how 18:1 ω 9 and myelination are related to behavioral development, and to elucidate the detailed relationships between fatty acids and brain development.

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